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(54) **Method and kit for identifying Phalaenopsis varieties**

(57) A method and a kit for identifying Phalaenopsis varieties are disclosed. The kit for identifying Phalaenopsis varieties of the present invention comprises: at least one microsatellite primer pair, wherein each of the microsatellite primer pair comprises: nucleotides with 75-100% identity to the sequences represented by the SEQ ID NOs: 1 to 72.

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Description

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefits of the Taiwan Patent Application Serial Number 100130368, filed on August 24, 2011, the subject matter of which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0002] The present invention relates to a method and a kit for identifying Phalaenopsis varieties. More specifically, the present invention relates to a method and a kit for identifying Phalaenopsis varieties by using microsatellite primer pairs.

2. Description of Related Art

[0003] Phalaenopsis is one of the top export floricultural goods from Taiwan. The export value of Phalaenopsis was about 99 million dollars in 2011, occupying 58% of the total export value for export flowers in Taiwan. At present, Taiwan has the vast Phalaenopsis orchid hybrids in the world, and has world-leading cultivation skills and breeding technique for modem, novel varieties bring about the great success. Therefore, Phalaenopsis is one of the most economically important floral crops in Taiwan; its economic value is most observable in areas such as commercial flask seedling, Phalaenopsis plantlet, pot flower, and cut flower.

[0004] The considerable number of Phalaenopsis varieties makes it necessary to identify the Phalaenopsis varieties for existing varieties, rare varieties, even the new varieties so as to distinguish the Phalaenopsis varieties and its economic value. In tradition, the main method used for identifying the Phalaenopsis varieties is based on the appearance , including flower color, pattern, flower size, flower count, numbers of spike, branching, stem height, etc. Nevertheless, it usually faces the problems about the serious mutations on flower shape and color of Phalaenopsis; or the pattern features affected by environment factors and the developmental stage of plants easily while using the traditional identification method. Meanwhile, not only do the mutations exist in the same variety, but also the mimic appearance exists in different varieties. Hence, resulting in the errors showed on the identification results. Besides, the traditional method is hard to apply on distinguishing the Phalaenopsis plantlet, and the time needed from plantlet to flowering is longer in Phalaenopsis. As a result, 1-3 years of waiting time is required before Phalaenopsis identification can begin.

[0005] Therefore, there is an urgent need to develop a method and a kit for identifying Phalaenopsis varieties, in hope that Phalaenopsis variety can be identified quicker at the early developmental stage; and infringement or tortious use of the variety can be reduced in order to protect the breeder's rights.

SUMMARY OF THE INVENTION

[0006] The object of the present invention is to provide a kit for identifying Phalaenopsis varieties so as to identify the Phalaenopsis varieties.

[0007] Another object of the present invention is to provide a method for identifying Phalaenopsis varieties so that Phalaenopsis variety can be identified quicker at the early developmental stage.

[0008] To achieve the aforementioned objects, the present invention provides a kit for identifying Phalaenopsis varieties, comprising: at least one microsatellite primer pair, wherein each of the microsatellite primer pair comprises: nucleotides with 75-100% identity to the sequences represented by a primer pair selected from the following groups: a first primer pair comprises the sequences represented by SEQ ID NO: 1 and SEQ ID NO: 2; a second primer pair comprises the sequences represented by SEQ ID NO: 3 and SEQ ID NO: 4; a third primer pair comprises the sequences represented by SEQ ID NO: 5 and SEQ ID NO: 6; a fourth primer pair comprises the sequences represented by SEQ ID NO: 7 and SEQ ID NO: 8; a fifth primer pair comprises the sequences represented by SEQ ID NO: 9 and SEQ ID NO: 10; a sixth primer pair comprises the sequences represented by SEQ ID NO: 11 and SEQ ID NO: 12; a seventh primer pair comprises the sequences represented by SEQ ID NO: 13 and SEQ ID NO: 14; an eighth primer pair comprises the sequences represented by SEQ ID NO: 15 and SEQ ID NO: 16; a ninth primer pair comprises the sequences represented by SEQ ID NO: 17 and SEQ ID NO: 18; a tenth primer pair comprises the sequences represented by SEQ ID NO: 19 and SEQ ID NO: 20; an eleventh primer pair comprises the sequences represented by SEQ ID NO: 21 and SEQ ID NO: 22; a twelfth primer pair comprises the sequences represented by SEQ ID NO: 23 and SEQ ID NO: 24; a thirteenth primer pair comprises the sequences represented by SEQ ID NO: 25 and SEQ ID NO: 26; a fourteenth primer pair comprises the sequences represented by SEQ ID NO: 27 and SEQ ID NO: 28; a fifteenth primer pair comprises

the sequences represented by SEQ ID NO: 29 and SEQ ID NO: 30; a sixteenth primer pair comprises the sequences represented by SEQ ID NO: 31 and SEQ ID NO: 32; a seventeenth primer pair comprises the sequences represented by SEQ ID NO: 33 and SEQ ID NO: 34; an eighteenth primer pair comprises the sequences represented by SEQ ID NO: 35 and SEQ ID NO: 36; a nineteenth primer pair comprises the sequences represented by SEQ ID NO: 37 and SEQ ID NO: 38; a twentieth primer pair comprises the sequences represented by SEQ ID NO: 39 and SEQ ID NO: 40; a twenty-first primer pair comprises the sequences represented by SEQ ID NO: 41 and SEQ ID NO: 42; a twenty-second primer pair comprises the sequences represented by SEQ ID NO: 43 and SEQ ID NO: 44; a twenty-third primer pair comprises the sequences represented by SEQ ID NO: 45 and SEQ ID NO: 46; a twenty-fourth primer pair comprises the sequences represented by SEQ ID NO: 47 and SEQ ID NO: 48; a twenty-fifth primer pair comprises the sequences represented by SEQ ID NO: 49 and SEQ ID NO: 50; a twenty-sixth primer pair comprises the sequences represented by SEQ ID NO: 51 and SEQ ID NO: 52; a twenty-seventh primer pair comprises the sequences represented by SEQ ID NO: 53 and SEQ ID NO: 54; a twenty-eighth primer pair comprises the sequences represented by SEQ ID NO: 55 and SEQ ID NO: 56; a twenty-ninth primer pair comprises the sequences represented by SEQ ID NO: 57 and SEQ ID NO: 58; a thirtieth primer pair comprises the sequences represented by SEQ ID NO: 59 and SEQ ID NO: 60; a thirty-first primer pair comprises the sequences represented by SEQ ID NO: 61 and SEQ ID NO: 62; a thirty-second primer pair comprises the sequences represented by SEQ ID NO: 63 and SEQ ID NO: 64; a thirty-third primer pair comprises the sequences represented by SEQ ID NO: 65 and SEQ ID NO: 66; a thirty-fourth primer pair comprises the sequences represented by SEQ ID NO: 67 and SEQ ID NO: 68; a thirty-fifth pair comprises the sequences represented by SEQ ID NO: 69 and SEQ ID NO: 70; and a thirty-sixth primer pair comprises the sequences represented by SEQ ID NO: 71 and SEQ ID NO: 72.

[0009] Thus, particularly a kit of the invention for identifying a Phalaenopsis variety comprises at least one microsatellite primer pair, wherein said primer pair is selected from a primer pair of SEQ ID NO: 1 and SEQ ID NO: 2; a primer pair of SEQ ID NO: 3 and SEQ ID NO: 4; a primer pair of SEQ ID NO: 5 and SEQ ID NO: 6; a primer pair of SEQ ID NO: 7 and SEQ ID NO: 8; a primer pair of SEQ ID NO: 9 and SEQ ID NO: 10; a primer pair of SEQ ID NO: 11 and SEQ ID NO: 12; a primer pair of SEQ ID NO: 13 and SEQ ID NO: 14; a primer pair of SEQ ID NO: 15 and SEQ ID NO: 16; a primer pair of SEQ ID NO: 17 and SEQ ID NO: 18; a primer pair of SEQ ID NO: 19 and SEQ ID NO: 20; a primer pair of SEQ ID NO: 21 and SEQ ID NO: 22; a primer pair of SEQ ID NO: 23 and SEQ ID NO: 24; a primer pair of SEQ ID NO: 25 and SEQ ID NO: 26; a primer pair of SEQ ID NO: 27 and SEQ ID NO: 28; a primer pair of SEQ ID NO: 29 and SEQ ID NO: 30; a primer pair of SEQ ID NO: 31 and SEQ ID NO: 32; a primer pair of SEQ ID NO: 33 and SEQ ID NO: 34; a primer pair of SEQ ID NO: 35 and SEQ ID NO: 36; a primer pair of SEQ ID NO: 37 and SEQ ID NO: 38; a primer pair of SEQ ID NO: 39 and SEQ ID NO: 40; a primer pair of SEQ ID NO: 41 and SEQ ID NO: 42; a primer pair of SEQ ID NO: 43 and SEQ ID NO: 44; a primer pair of SEQ ID NO: 45 and SEQ ID NO: 46; a primer pair of SEQ ID NO: 47 and SEQ ID NO: 48; a primer pair of SEQ ID NO: 49 and SEQ ID NO: 50; a primer pair of SEQ ID NO: 51 and SEQ ID NO: 52; a primer pair of SEQ ID NO: 53 and SEQ ID NO: 54; a primer pair of SEQ ID NO: 55 and SEQ ID NO: 56; a primer pair of SEQ ID NO: 57 and SEQ ID NO: 58; a primer pair of SEQ ID NO: 59 and SEQ ID NO: 60; a primer pair of SEQ ID NO: 61 and SEQ ID NO: 62; a primer pair of SEQ ID NO: 63 and SEQ ID NO: 64; a primer pair of SEQ ID NO: 65 and SEQ ID NO: 66; a primer pair of SEQ ID NO: 67 and SEQ ID NO: 68; a primer pair of SEQ ID NO: 69 and SEQ ID NO: 70; and a primer pair of SEQ ID NO: 71 and SEQ ID NO: 72, or a primer pair having at least 75% identity thereto.

[0010] A primer pair present in the kit may therefore have at least 80, 85, 90, 95, 96, 97, 98 or 99% sequence identity with any one of the primer pairs set out above.

[0011] Identity may be determined using the Bestfit program of the Genetics Computer Group Version 10 software package from the University of Wisconsin. The Pogram uses the local hand algorithm of Smith and Waterman with the default values: Gap creation penalty=8, Gap extension penalty=2, Average match=2.912, Average mismatch=2.003.

[0012] Thus, as described above, a primer pair typically comprises two primers and as discussed above a primer pair in the kit of the invention may consist of two primers having the specific sequences as set out in the pairs of SEQ ID NOs listed above, or may have at least 75% identity to any of those sequences. Thus, in any primer pair, either or both primers may have at least 75 % identity to any of the primers in any of the specific primer pair sequences. Hence, for example for a primer pair of SEQ ID NO. 1 and SEQ ID NO. 2, the kit may comprise primers with these specific sequences or may comprise one primer with at least 75% identity to SEQ ID NO. 1 and the other primer having the sequence of SEQ ID NO. 2, or may comprise one primer having the sequence of SEQ ID NO.1 and the other primer with at least 75% identity to SEQ ID NO. 2 or may comprise one primer with at least 75% identity to SEQ ID NO. 1 and the other primer with at least 75% identity to SEQ ID NO. 2.

[0013] The kit of the invention may comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 or 35 primer pairs.

[0014] Particularly, the kit may comprise primer pairs of SEQ ID NO. 1 and SEQ ID NO. 2, SEQ ID NO. 3 and SEQ ID NO. 4, SEQ ID NO. 5 and SEQ ID NO. 6, SEQ ID NO. 7 and SEQ ID NO. 8, SEQ ID NO. 13 and SEQ ID NO. 14, SEQ ID NO. 31 and SEQ ID NO. 32 and SEQ ID NO. 37 and SEQ ID NO. 38 or primer pairs having at least 75% identity thereto.

[0015] Typically, one of the primers in the primer pair is a forward primer and the other primer is a reverse primer.

[0016] Furthermore, the present invention provides a method by using the aforementioned kit for identifying Phalaenopsis varieties, comprising the following steps: (A) providing a tissue sample of the to be tested Phalaenopsis variety, and isolating the genomic DNA from the tissue sample; (B) obtaining the genotype of a tested Phalaenopsis variety by using at least one microsatellite primer pair to amplify the genomic DNA of the tissue sample and (C) comparing the genotype obtained from the tested Phalaenopsis variety with a known database of the Phalaenopsis genotype to identify the variety of the tested sample.

[0017] Specifically, the invention provides a method for identifying a Phalaenopsis variety comprising

A) providing a tissue sample of a Phalaenopsis variety and isolating genomic DNA therefrom,
B) determining the genotype of said Phalaenopsis variety using at least one microsatellite primer pair as set out above to amplify the genomic DNA and
C) comparing said genotype with a known database of Phalaenopsis variety genotypes to identify the variety of the tested Phalaenopsis.

[0018] In the kit and the method for identifying Phalaenopsis varieties of the present invention, it only requires a very small amount of genomic DNA isolated from plant tissue to identify the Phalaenopsis varieties quickly by using microsatellite primer pair. Compared with the DNA molecular marker of the techniques such as amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), and restriction fragment length polymorphism (RFLP), the microsatellite sequence of the present invention has the advantages of being handled relatively quickly and simplistically (only 3 to 7 days is needed for the identification), high polymorphism, high reproducibility, and applying to the automatic analysis with high throughput. Besides, the kit and the method for identifying Phalaenopsis varieties of the present invention are not affected by the growth environment of plants. Therefore, the kit and the method can be applied for detecting the non-flowered plants, plantlet, or flask seedling, and for identifying the Phalaenopsis varieties at the early developmental stage. Furthermore, the kit and the method for identifying Phalaenopsis varieties provided by the present invention can give a unique DNA molecular code for every Phalaenopsis variety, increasing the speed and the accuracy for identifying Phalaenopsis varieties.

[0019] The tissue sample used in the method may be root, stem, flower or leaves

[0020] In the kit and the method for identifying Phalaenopsis varieties of the present invention, the better, used microsatellite primer pairs is a set of the primer pairs which comprises any one nucleotide sequence selected from the group consisting of the first primer pair to the thirty-sixth primer pair. In addition, the 5' end or the 3' end of the sequence of these primer pairs can further add several nucleotides (1 to 15 different). For example, at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 nucleotides may be added to either or both of the 5' and/or 3' ends of a primer in a primer pair. Preferably, the best, used primer pairs are a set of the primer pairs which is selected from the group consisting of the first primer pair to the thirty-sixth primer pair.

[0021] Moreover, in the kit and the method for identifying Phalaenopsis varieties of the present invention, at least one microsatellite primer pair includes a forward microsatellite primer and a reverse microsatellite primer. Preferably, one of the 5' ends of the forward microsatellite primer or the reverse microsatellite primer labeled with a fluorescent reagent. After the primer pair labeling with the fluorescent reagent and then obtaining a amplification product by polymerase chain reaction (PCR), the genotype of Phalaenopsis varieties can be determined by DNA analyzer. A preferred arrangement is that different microsatellite primer pairs labeled with different fluorescent reagents and the amplification products with different fluorescent reagents labeled are mixed into the same tube before conducting the genotyping analysis. The fluorescent reagent labeled at either the forward primer or the reverse primer can be a common fluorescent labeling reagent used in this technical field, such as HEX, FAM, and NED.

[0022] Also, in the method for identifying Phalaenopsis varieties of the present invention, step (B) involves using a DNA analysis technique commonly known in this field of technology to analyze the genomic DNA of the tissue sample; a preferred embodiment of this aspect of the invention is to use polymerase chain reaction (PCR) to analyze the genomic DNA of a tissue sample, using at least one microsatellite primer pair. In addition, the size of the amplified fragment can be used to differentiate Phalaenopsis varieties.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023]

FIG. 1 is the preferred embodiment of the present invention showing the results of *Dtps.* Taida Firebird 'Taida Red Rose' and *Dtps.* Sogo Meili 'SOGO F1751' by DNA analyzer; and

FIG. 2 is the preferred embodiment of the present invention showing the result of similarity cluster analysis for identifying Phalaenopsis varieties by using microsatellite primer pairs.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0024] The present invention has been described in an illustrative manner, and it is to be understood that the terminology used is intended to be in the nature of description rather than of limitation. Many modifications and variations of the present invention are possible in light of the above teachings. Therefore, it is to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described.

<Isolating the genomic DNA from the tissue sample of Phalaenopsis variety>

[0025] Root, stem, leaf, or flower tissues of Phalaenopsis variety were collected for analyzing the genotypes of Phalaenopsis varieties. The tissues were ground into powder by a tissue grater or pulverized in liquid nitrogen, the genomic DNA was mini-extracted by use of the CTAB (cetyltrimethylammonium bromide) method or the BioKit plant genomic DNA purification kit and diluted to a concentration of 3 ng/μL and stored at -20°C waiting for use.

[0026] 51 Phalaenopsis varieties (as shown in the following Table 2) are collected for the present embodiment, for which applications for the plant breeder's rights are currently pending or already obtained in Taiwan. About 0.2 g leaf tissue of Phalaenopsis varieties are provided here for demonstration, which were ground into fine powder with the presence of liquid nitrogen. The plant DNAs were isolated using the BioKit Plant Genomic DNA Purification Kit (BioKit Biotechnology Incorporation) or the QIAGEN DNeasy Plant Mini Kit (QIAGENE), and were diluted to 3 ng/μL in concentration and stored at -20°C before use.

<Analyzing the genomic DNA via PCR>

[0027] First, the 5 μL genomic DNA (3 ng/μL) of the Phalaenopsis tissue sample was taken, and the 2.5 μL, 10X PCR buffer (VIOGENE); 2.5 μL, 1% Tween 20; 1.0 μL, 2.5 mM dNTP; 1.0 μL, 5 μmol forward primer of microsatellite primer (as shown in the following Table 1) labeled with a fluorescent reagent; 1.0 μL, 5 μmol reverse primer of microsatellite primer (as shown in the following Table 1 without labeling a fluorescent reagent); 0.2 μL, 5 U DNA polymerase (PureTaq DNA polymerase, VIOGENE) were added. The sterile water was added to count for 25 μL for the total volume and proceeded through the PCR amplification. Wherein the conditions of PCR amplification were as follows: first, reacted 5 minutes at 94°C; 40 cycles were repeated and each cycle was designed to react 30 seconds at 94°C; reacted 30 seconds at annealing temperature (as shown in the following Table 1); reacted 40 seconds at 72°C; finally, reacted 5 minutes at 72°C and the reaction stopping temperature was 4°C.

Table 1

Primer pair No.	SEQ ID NO:	Forward and reverse microsatellite primer sequence	Tm (°C)	Expected size (bp)	Fluorescent reagent
1	1	F: GCCCAATTTTCAGTTTTCCTTC	55	400	FAM/NED
	2	R: TTGCTTCATTGTTTTTCCTTTCAC			
2	3	F: TCTTTGTGTGTGTGTGCGTG	55	179	FAM
	4	R: GTGTCCCGTAGACTTCCCG			
3	5	F: GGCGAGCGATTGGTGTGC	55	283	FAM
	6	R: TTTTTTCCTCATTCGGTCAGGC			
4	7	F: CTTGAGCGGATTGGGTCTTA	51	268	HEX
	8	R: CACTCAGCCAACGCATCGAA			
5	9	F: GCAATTTGACTACCCCCTCTC	51	167	TET
	10	R: GCATGGATTAACCGTTTTTCTT			
6	11	F: TTCGGACTACAAGGCGTACC	55	236	FAM
	12	R: CATTTGGCTCAGGCTCAGTAC			
7	13	F: CTCATTCGGTCAGGCATTTC	55	172	HEX
	14	R: AGCTGCTTCCCAAGTGATTC			
8	15	F: GAATGAGGGCACCAAATCT	49	338	HEX/NED
	16	R: TCCATTAACCCCGAGAAAAA			
9	17	F: GGAACCAACAAGAAGAAGAAGC	51	232	FAM
	18	R: GCATCGCAGAGAACAGAGAGC			
10	19	F: GGGATGGAGGGATTTGAGAT	50	287	FAM
	20	R:AAGCACAGGAAACGCAAACT			

(continued)

Primer pair No.	SEQ ID NO:	Forward and reverse microsatellite primer sequence	Tm (°C)	Expected size (bp)	Fluorescent reagent
11	21	F: GAGCCCGATCACAACGACC	55	325	FAM
	22	R: GTGACTCCGTTCCATGCCTC			
12	23	F: AAGATGGGTAGTATGTGAGAATGC	51	232	HEX
	24	R: ATCAGCCACAAAGCAGAAGC			
13	25	F: GGAAGCAGGCAAAGGTATGA	51	218	FAM
	26	R: TTGCTGTCTCCCTCATCTGC			
14	27	F: AGCCTACCTGATTCGCCATA	51	211	HEX
	28	R: CCCATTCATCCCCTCTCTCT			
15	29	F: ACACTCCCTGCCAATGGTAA	51	250	NED
	30	R: TCCATTTCCTTCATTTTGTGC			
16	31	F: GGTCAGCCTCCTTCATCAGA	50	229	HEX
	32	R: TGGTCATGTGGTGTGTTTTTA			
17	33	F: TAGTGGTGGTGGCGATGATA	50	166	FAM
	34	R: CTAAATAATGCCAAGGGGTAACT			
18	35	F: GTAATCACAACTAAACAGGGCTAA	49	240	HEX
	36	R: ATGATGATGAGGAGGAGTAGATTC			
19	37	F: TGGTCTCTGTCGTCACTTGG	50	219	FAM
	38	R: AAGAATTACACCGCCGATCA			
20	39	F: CATTAGTGGTCTTTCCCTGTG	47	300	FAM
	40	R: AAGAGTGGATGTTTTAGTTTCG			
21	41	F: GCTTTCTGCTTATCGTTCCA	49	247	FAM
	42	R: GCTTACAGTGGACGGGCT			
22	43	F: TGGAGGGTTAGAAGCAAGGA	50	223	FAM
	44	R:TCATCATCCCTCTTCCTCGT			
23	45	F: GTTTATCTTCATGTGGTCCTCG	51	256	HEX
	46	R: CTTACTTGTCCACCCCCATT			
24	47	F: GGCTCACCTTCCTTCTCCTT	51	260	NED
	48	R:GCTAAACCCTAAACCCTTGTGT			
25	49	F: CAACTCCCAAAGCCTCAACT	50	185	HEX
	50	R: GACCCACACCATTCCATCAC			
26	51	F: CTAACTCTCTTTCCTTGCTCCTT	50	195	FAM
	52	R: CGTTTCTTCCTCTCTCACACTC			
27	53	F: TTGGATGGTAGGTATGAGTATGC	50	249	FAM
	54	R: TGATTGTTCTTCCCGTCTGTT			
28	55	F: CCTCGTGCTTTCCTGTTCAT	50	215	FAM
	56	R: AGTTTTGGGTGCTGCTATGC			
29	57	F: ATGAATCGTTTGGTATGTATGTGA	50	220	NED
	58	R: ATTGTTCATTGCTGCTGCTG			
30	59	F: AAGTTATCCAAATGACCCCAG	50	272	FAM
	60	R: AAACTTCGGTGCCTGAGAGA			
31	61	F: CCGCTCTTCCAGTTTCGTT	52	187	NED
	62	R: AAATCATCTTAGGAGCACCATCA			
32	63	F: AAAAGGAGAAGCGAGGAAGG	50	163	HEX
	64	R: CTTCCTCTTCGCTTGGTGA			
33	65	F: ATGGAGGAAAGTGATAGTAAGAGAA	50	151	FAM
	66	R: GAGGAGAGTGTGCTAAGTGGTG			
34	67	F: AAGATTTTTGTGGTGGGAA	50	171	NED
	68	R: ACTCAATAGGTCGCAATGG			
35	69	F: CACGTTTCTCTACGGGGAC	50	262	HEX

(continued)

Primer pair No.	SEQ ID NO:	Forward and reverse microsatellite primer sequence	Tm (°C)	Expected size (bp)	Fluorescent reagent
	70	R: CTGCCTTTCTGTTGAACTCC			
36	71	F: GCGTGTGACTGATGGAAGAAG	50	357	FAM
	72	R: TCCCACCGAGTGGAGAATAC			

[0028] Electrophoresis analysis was performed on a 2 to 3 % agarose gel, confirming whether the products amplified via PCR by the microsatellite primer pairs existed or not. The amplified PCR products with different fluorescent reagents labeled were mixed, and sent to any Biotechnology Company to identify the genotypes of Phalaenopsis varieties derived from microsatellite molecular markers by using DNA analyzer. Finally, the allele sizes were scored using Peak Scanner or GeneMapper software to analyze the genotypes of Phalaenopsis varieties.

[0029] In the present embodiment, the 19 sets of the microsatellite primer pairs (as shown in the following Table 3) were used to perform PCR amplification and analysis with regard to the 51 Phalaenopsis varieties (as shown in the following Table 2). Product electrophoresis was carried out on a 3 % agarose gel, for the purpose of checking whether the products amplified via PCR existed in the range of expected size (as shown in the aforementioned Table 1). The 2 to 3 amplified PCR products labeled with different fluorescent reagents were mixed in the same tube, and sent to any Biotechnology Company to identify the genotypes of the 51 Phalaenopsis varieties derived from the 19 sets of the microsatellite molecular markers by DNA analyzer (ABI PRISM 3730 DNA analyzer). The results for analyzing *Dtps.* Taida Firebird 'Taida Red Rose' by using the first primer pair and the ninth primer pair, and for analyzing *Dtps.* Sogo Meili 'SOGO F1751' by using the tenth primer pair and the fourteenth primer pair via DNA analyzer were shown in Figure 1.

[0030] The identified results were recored via Peak Scanner software and the genotypes obtained via analysis by the 19 sets of the microsatellite primer pairs for every Phalaenopsis variety afterward, as shown in the following Table 2 (only exhibited the analytical results by the first, the second, and the third primer pairs). According to the allele observed in the Phalaenopsis variety: the specific alleles were represented as "1" (present) and "0" (absent). The similarity analysis was performed to analyze the varieties using NYSYS software and the results are shown in Figure 2. The 1 to 51 numbers shown in Figure 2 represent the 51 Phalaenopsis varieties. The Figure 2 are the results for identifying the genotypes using 19 sets of the microsatellite molecular markers, and it can discriminate the 51 Phalaenopsis varieties which were applied for registration in Taiwan or granted the plant variety rights in Taiwan. Therefore, the results demonstrated that the microsatellite molecular markers labeled with fluorescent reagents of the present invention can identify the Phalaenopsis varieties successfully.

Table 2

		Amplified product size (bp)		
No.	**Phalaenopsis varieties**	**The first primer pair**	**The second primer pair**	**The third pair primer**
1	*P.* Yu Pin pearl 'YPM131'	325, 329	166, 177, 200	N[a]
2	*P.* Taihort Gem 'TSC 13 8'	320, 328	142, 165	291
3	*P.* Sogo Imp 'SOGO F-940'	320	200	281,287
4	*P.* Join Grace 'TH.288-4'	320, 325, 320, 325,	164, 190	299
5	*P.* Sogo Firework 'SOGO F-802'	320, 343	164, 198	303, 308
6	*P.* Sogo Gold 'SOGO F-1046'	328337	165,200	296,299
7	*P.* Tai Lin Prince 'Bei ji guang V265'	320, 337	N	299, 305
8	*P.* Tai Ling Queen 'Queen V6'	319, 325, 337	177	N
9	*P.* Dou-dii Pride 'Mei Dar Red Star'	320, 328	164, 166	283,299
10	*P.* Sogo Gold 'SOGO F1047'	309, 328, 337	165, 177	299
11	*P.* Classic Beauty 'Color Butterfly'	318, 343	169, 177	283,298
12	*P.* Sogo Yukidian 'Shiuh-Dong Whishkey'	320, 337	177	299

(continued)

No.	Phalaenopsis varieties	Amplified product size (bp) The first primer pair	The second primer pair	The third pair primer
13	*P*. Lawrence of Arabia 'NCYU Sandy'	318, 337	164, 177 287	281, 285,
14	*P*. Ho's Colourful Bubbles 'The Pride of Taiwan'	318, 320, 328	182	283, 287, 305
15	*P*. Tainung No. 1 'Pixie'	309, 343	142	305, 307
16	*Dtps.* Sogo Romantic 'SOGO F-982'	309, 319, 327, 329	165	283, 295, 308
17	*Dtps.* Bread Rose 'Lih Jiang Firebird'	328, 337	165	283, 291, 299
18	*Dtps.* Sogo Yoshida 'SOGO F-1302'	319, 337	176	283
19	*Dtps.* Lepoard Prince 'SOGOF-1138'	320, 328	165, 176	291 299 307 299,
20	*Dtps.* Sogo Pride 'SOGOF-1016'	318, 337	176	285, 293, 299
21	*Dtps.* Sogo vivien 'SOGO F-858'	320, 328	142, 165	291, 303
22	*Dtps.* Nobby's Pink Lady 'Pingtung Queen'	320, 327	176, 198	282
23	P. Sogo Muyudian 'LW9441 ' 'Melor CL331'	309, 320, 337	166, 177	297, 299
24	P. Unimax Glory 'LW9509' 'White Ribbon CL369'	318, 320, 337	166, 177	296
25	P. Unimax Moonlight 'Fortune CL577'	337	184, 200	283, 287, 289, 299
26	P. Unimax Sakurahime 'Princess Sakura CL805'	327	177, 200	N
27	*Dtps.* Unimax Cradle 'SWR9501' 27 'Lullaby CL904'	310, 337	177, 180	N
28	*Dtps.* Sogo Moonhalo 'Sogo F-1061'	322, 337	165, 177, 200	283, 291, 283, 291, 299
29	*Dtps.* Sogo Alice 'Sogo F-1199'	320, 339	165, 177	285, 295, 303 295,
30	*Dtps.* Sogo Gumbo 'Sogo F-981'	309, 320, 327	165, 177	283,295
31	*Dtps.* Sogo Weddung 'Sogo F-879'	366	142, 165, 177	285,295
32	*Dtps.* Sogo Gotris 'Sogo F-1307'	325, 339	142	298
33	*Dtps.* Leopard Prince 'SOGO F-977'	320, 329	161, 200	283, 295, 307 295,
34	*P*. Sogo Golden Timothy 'Sogo F-1034'	314, 337, 366	168	281, 305
35	*P*. Taisuco Eros 'Pink Butterfly'	320, 329, 339	165, 176, 185, 200	283, 291, 303
36	*P*. Taisuco Pioneer 'Vanguard'	317, 327, 336	165, 177	285, 303
37	*P*. Taisuco Snowing 'TSC 139'	309	177	293, 303

(continued)

No.	Phalaenopsis varieties	Amplified product size (bp) The first primer pair	The second primer pair	The third pair primer
38	*P*. Taisuco Stellar 'Red Pearl'	328, 339	142,200	291, 303
39	*P*. Sogo Fairyland 'Sogo F-842'	320, 337, 343	141, 176	297, 305
40	*P*. SOGO Venis 'SOGO F1314'	337	180	289, 305
41	*Dtps.* Sogo Gotris 'SOGO F1248'	319 325 339 325,	141, 176	297
42	*Dtps*. Sogo Meili 'SOGO F1751'	319, 328	176	293,295
43	*Dtps.* Sogo Melody 'SOGO F1951'	318, 322, 329, 337	165, 186	283,295
44	*P*. Sogo Lawrence 'SOGO F-1982'	318, 325	176, 200	283, 291, 299
45	*Dtps.* Sogo Passat 'Sogo F-1383'	328, 336	176, 200	283, 291, 304 291,
46	*P*. Taida Smile 'Taida Little Green'	316, 337	200	283, 295, 305
47	*P*. Taida Lovely 'Taida Pink Swan'	318, 327	165, 176	296, 299
48	*Dtps.* Taida Firebird 'Taida Red Rose'	316, 329 165,	176	291, 303
49	*Dtps.* Hsinying Mount 'Taida Snow Peach'	320, 337	166, 176, 185,200	296, 299, 312
50	*Dtps.* SOGO Breeze 'SOGO F1621'	320, 339, 365' 339,	142, 176	309
51	*P*. Sogo Relex 'SOGO F1661'	323, 337	165	287, 312

[a]N: represents the Phalaenopsis variety did not have PCR amplified product by the microsatellite primer pair.

[0031] In addition, the frequency and the number of the alleles existed in the 51 Phalaenopsis varieties by each microsatellite primer pair were analyzed, and the PIC value that represents the discrimination power of the microsatellite molecular marker was calculated according to the genotyping results. The PIC values of the 19 sets of the microsatellite molecular markers are ranging from 0.62 (the sixth primer pair) to 0.95 (the twenty-third primer pair). In the statistics of unique genotype number, 49 different unique genotypes were obtained in the 51 varieties by using the seventh primer pair. It represents that one set of the microsatellite primer pair- the seventh primer pair can identify 49 varieties (as shown in the following Table 3). Hence, the results demonstrated that the microsatellite molecular markers of the present invention have high identification efficacy to distinguish the Phalaenopsis varieties.

Table 3

Microsatellite primer pair	Fluorescent reagent	Amplified product length (bp)	Different genotype number[a]	Allele number[a]	PIC value[b]
The first primer pair	FAM/NED	309-366	43	20	0.9
The second primer pair	HEX	141-200	34	18	0.88
The third primer pair	FAM	281-312	40	20	0.92
The fourth primer pair	HEX	241-386	31	24	0.92

(continued)

Microsatellite primer pair	Fluorescent reagent	Amplified product length (bp)	Different genotype number[a]	Allele number[a]	PIC value[b]
The fifth primer pair	TET	150-269	21	18	0.87
The sixth primer pair	FAM	208-236	6	5	0.62
The seventh primer pair	HEX	135-171	49	31	0.94
The eighth primer pair	HEX/NED	309-345	43	21	0.92
The ninth primer pair	FAM	183-233	42	27	0.88
The tenth primer pair	FAM	251-280	18	11	0.74
The eleventh primer pair	FAM	291-346	14	12	0.73
The twelfth primer pair	HEX	214-256	44	23	0.91
The thirteenth primer pair	FAM	192-242	39	19	0.86
The fourteenth primer pair	HEX	185-260	48	33	0.94
The fifteenth primer pair	NED	212-315	32	26	0.93
The sixteenth primer pair	HEX	226-280	45	27	0.92
The nineteenth primer pair	FAM	194-219	34	14	0.89
The twenty-third primer pair	HEX	198-255	47	32	0.95
The twenty-fourth primer pair	NED	219-330	35	24	0.9

[a]Different genotype number and [a]Allele number: represent the genotype number and allele number obtained in the 51 Phalaenopsis varieties by each microsatellite primer pair.
[b]PIC value: Polymorphism Information Content, represents the discrimination power of microsatellite molecular marker; the PIC value is larger, and the microsatellite molecular marker is more discriminative.

[0032] According to the results of the abovementioned Table 1 to Table 3 and Figure 1 to Figure 2, the microsatellite primer pair of the present invention has excellent identifying ability; the abovementioned Phalaenopsis varieties can be identified by using only a few sets of the microsatellite primer pairs. Moreover, identifying varieties can be performed without Phalaenopsis flowering. Therefore, the present invention can be used in the non-flowering plantlet for identifying at the early developmental stage as well as increasing the accuracy for identifying Phalaenopsis varieties.

[0033] On the other hand, the present invention providing the kit and the method for identifying Phalaenopsis varieties can give a unique DNA molecular code for every Phalaenopsis variety, construct a DNA database and reduce the possibility of the varieties suffered from infringement and tortious actions.

[0034] Here, some Phalaenopsis varieties were analyzed by using the microsatellite primer pairs (the first primer pair, the second primer pair, the third primer pair, the fourth primer pair, the seventh primer pair, the sixteenth primer pair, and the nineteenth primer pair) of the present invention to obtain the DNA molecular code of the genotype of Phalaenopsis varieties. The results are shown in the following Table 4.

Table 4

Varieties	The first primer pair		The second primer pair		The third primer pair		The fourth primer pair		The seventh primer pair		The sixteenth primer pair		The nineteenth primer pair		Code
P. Join Grace 'TH. 288-4'	320, 325, 343	Q	164, 190	G	299	F	276, 282	J	153, 155	P	269, 271	Q	200, 213	G	QGF JPQG
P. Tai Lin Prince 'Bei ji guang V265'	320, 337	G	N	M	299, 305	L	241	A	144, 155, 161	G	257, 265, 269, 277	K	200, 213	G	GML AGKG
P. Tai Ling Queen 'Queen V6'	319, 325, 337	O	177	D	N	R	241, 251	B	146, 155, 163	K	265, 271, 279	O	200, 214, 216	H	ODR BKOH
P. Sogo Yukidian 'Shiuh-Dong Whishkey'	320, 337	G	177	D	299	F	241	A	153, 155, 164	Q	269, 271, 280	R	200	C	GDF AQRC

[0035] For example, when analyzing by using the first primer pair as the microsatellite primer pair, the letter "G" can represent genotype (320, 337); when analysis is using the second primer pair as the microsatellite primer pair, the letter "D" and "G" can represent genotype (177) and (164, 190) respectively. In other words, the different letters are obtained based on different genotypes by the analysis of each primer pair, and every analyzing result of primer pair is corresponding to a letter separately. In the present embodiment, identification by using 7 sets of microsatellite primer pairs can obtain the DNA molecular code composed of 7 letters. For instance, the results for identifying P. Join Grace 'TH.288-4' and *P.* Tai Lin Prince 'Bei ji guang V265' can show the unique DNA molecular codes- QGFJPQG and GMLAGKG, respectively by using 7 sets of the microsatellite primer pairs.

[0036] The more sets of microsatellite primer pairs are used in analysis, the more letters can be obtained to compose a specific molecular code. Based on this unique DNA molecular code, a DNA database of Phalaenopsis varieties can be constructed to reduce infringement or tortious use of the variety in order to protect breeder's rights.

[0037] Although the present invention has been explained in relation to its preferred embodiment, it is to be understood that many other possible modifications and variations can be made without departing from the spirit and scope of the invention as hereinafter claimed.

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<120> Method and kit for identifying Phalaenopsis varieties

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Claims

1. A kit for identifying Phalaenopsis varieties, comprising:

at least one microsatellite primer pair, wherein each of the microsatellite primer pair comprises: nucleotides with 75-100% identity to the sequences represented by a primer pair selected from the following groups:

a first primer pair comprising the sequences represented by SEQ ID NO: 1 and SEQ ID NO: 2;
a second primer pair comprises the sequences represented by SEQ ID NO: 3 and SEQ ID NO: 4;
a third primer pair comprises the sequences represented by SEQ ID NO: 5 and SEQ ID NO: 6;
a fourth primer pair comprises the sequences represented by SEQ ID NO: 7 and SEQ ID NO: 8;
a fifth primer pair comprises the sequences represented by SEQ ID NO: 9 and SEQ ID NO: 10;
a sixth primer pair comprises the sequences represented by SEQ ID NO: 11 and SEQ ID NO: 12;
a seventh primer pair comprises the sequences represented by SEQ ID NO: 13 and SEQ ID NO: 14;
a eighth primer pair comprises the sequences represented by SEQ ID NO: 15 and SEQ ID NO: 16;
a ninth primer pair comprises the sequences represented by SEQ ID NO: 17 and SEQ ID NO: 18;
a tenth primer pair comprises the sequences represented by SEQ ID NO: 19 and SEQ ID NO: 20;
an eleventh primer pair comprises the sequences represented by SEQ ID NO: 21 and SEQ ID NO: 22;
a twelfth primer pair comprises the sequences represented by SEQ ID NO: 23 and SEQ ID NO: 24;
a thirteenth primer pair comprises the sequences represented by SEQ ID NO: 25 and SEQ ID NO: 26;
a fourteenth primer pair comprises the sequences represented by SEQ ID NO: 27 and SEQ ID NO: 28;
a fifteenth primer pair comprises the sequences represented by SEQ ID NO: 29 and SEQ ID NO: 30;
a sixteenth primer pair comprises the sequences represented by SEQ ID NO: 31 and SEQ ID NO: 32;
a seventeenth primer pair comprises the sequences represented by SEQ ID NO: 33 and SEQ ID NO: 34;
an eighteenth primer pair comprises the sequences represented by SEQ ID NO: 35 and SEQ ID NO: 36;

a nineteenth primer pair comprises the sequences represented by SEQ ID NO: 37 and SEQ ID NO: 38;
a twentieth primer pair comprises the sequences represented by SEQ ID NO: 39 and SEQ ID NO: 40;
a twenty-first primer pair comprises the sequences represented by SEQ ID NO: 41 and SEQ ID NO: 42;
a twenty-second primer pair comprises the sequences represented by SEQ ID NO: 43 and SEQ ID NO: 44;
a twenty-third primer pair comprises the sequences represented by SEQ ID NO: 45 and SEQ ID NO: 46;
a twenty-fourth primer pair comprises the sequences represented by SEQ ID NO: 47 and SEQ ID NO: 48;
a twenty-fifth primer pair comprises the sequences represented by SEQ ID NO: 49 and SEQ ID NO: 50;
a twenty-sixth primer pair comprises the sequences represented by SEQ ID NO: 51 and SEQ ID NO: 52;
a twenty-seventh primer pair comprises the sequences represented by SEQ ID NO: 53 and SEQ ID NO: 54;
a twenty-eighth primer pair comprises the sequences represented by SEQ ID NO: 55 and SEQ ID NO: 56;
a twenty-ninth primer pair comprises the sequences represented by SEQ ID NO: 57 and SEQ ID NO: 58;
a thirtieth primer pair comprises the sequences represented by SEQ ID NO: 59 and SEQ ID NO: 60;
a thirty-first primer pair comprises the sequences represented by SEQ ID NO: 61 and SEQ ID NO: 62;
a thirty-second primer pair comprises the sequences represented by SEQ ID NO: 63 and SEQ ID NO: 64;
a thirty-third primer pair comprises the sequences represented by SEQ ID NO: 65 and SEQ ID NO: 66;
a thirty-fourth primer pair comprises the sequences represented by SEQ ID NO: 67 and SEQ ID NO: 68;
a thirty-fifth pair comprises the sequences represented by SEQ ID NO: 69 and SEQ ID NO: 70; and
a twenty-sixth primer pair comprises the sequences represented by SEQ ID NO: 71 and SEQ ID NO: 72.

2. The kit as claimed in claim 1, wherein each of the microsatellite primer pair is a set of the primer pairs, and the set of the primer pairs comprise: any one nucleotide sequence selected from the group consisting of the first primer pair to the thirty-sixth primer pair.

3. The kit as claimed in claim 1, wherein each of the microsatellite primer pair is a set of the primer pairs, and the set of the primer pairs are selected from the group consisting of the first primer pair to the thirty-sixth primer pair.

4. The kit as claimed in claim 1, wherein the at least one microsatellite primer pair comprises: a forward microsatellite primer and a reverse microsatellite primer.

5. The kit as claimed in claim 4, wherein the 5' end of the forward microsatellite primer is labeled with a fluorescent reagent.

6. The kit as claimed in claim 5, wherein the fluorescent reagent is HEX, FAM, or NED.

7. The kit as claimed in claim 4, wherein the 5' end of the reverse microsatellite primer is labeled with a fluorescent reagent.

8. The kit as claimed in claim 7, wherein the fluorescent reagent is HEX, FAM, or NED.

9. A method for identifying Phalaenopsis varieties, comprising the following steps:

(A) providing a tissue sample of a tested Phalaenopsis variety, and isolating the genomic DNA from the tissue sample;
(B) detecting the genotype of the tissue sample of the tested Phalaenopsis variety by using at least one microsatellite primer pair to obtain a genotype, wherein each of the primer pair comprises: a nucleotide with 75-100% identity to the sequences represented by a primer pair selected from the following groups:

a first primer pair comprises the sequences represented by SEQ ID NO: 1 and SEQ ID NO: 2;
a second primer pair comprises the sequences represented by SEQ ID NO: 3 and SEQ ID NO: 4;
a third primer pair comprises the sequences represented by SEQ ID NO: 5 and SEQ ID NO: 6;
a fourth primer pair comprises the sequences represented by SEQ ID NO: 7 and SEQ ID NO: 8;
a fifth primer pair comprises the sequences represented by SEQ ID NO: 9 and SEQ ID NO: 10;
a sixth primer pair comprises the sequences represented by SEQ ID NO: 11 and SEQ ID NO: 12;
a seventh primer pair comprises the sequences represented by SEQ ID NO: 13 and SEQ ID NO: 14;
an eighth primer pair comprises the sequences represented by SEQ ID NO: 15 and SEQ ID NO: 16;
a ninth primer pair comprises the sequences represented by SEQ ID NO: 17 and SEQ ID NO: 18;
a tenth primer pair comprises the sequences represented by SEQ ID NO: 19 and SEQ ID NO: 20;
an eleventh primer pair comprises the sequences represented by SEQ ID NO: 21 and SEQ ID NO: 22;

a twelfth primer pair comprises the sequences represented by SEQ ID NO: 23 and SEQ ID NO: 24;
a thirteenth primer pair comprises the sequences represented by SEQ ID NO: 25 and SEQ ID NO: 26;
a fourteenth primer pair comprises the sequences represented by SEQ ID NO: 27 and SEQ ID NO: 28;
a fifteenth primer pair comprises the sequences represented by SEQ ID NO: 29 and SEQ ID NO: 30;
a sixteenth primer pair comprises the sequences represented by SEQ ID NO: 31 and SEQ ID NO: 32;
a seventeenth primer pair comprises the sequences represented by SEQ ID NO: 33 and SEQ ID NO: 34;
an eighteenth primer pair comprises the sequences represented by SEQ ID NO: 35 and SEQ ID NO: 36;
a nineteenth primer pair comprises the sequences represented by SEQ ID NO: 37 and SEQ ID NO: 38;
a twentieth primer pair comprises the sequences represented by SEQ ID NO: 39 and SEQ ID NO: 40;
a twenty-first primer pair comprises the sequences represented by SEQ ID NO: 41 and SEQ ID NO: 42;
a twenty-second primer pair comprises the sequences represented by SEQ ID NO: 43 and SEQ ID NO: 44;
a twenty-third primer pair comprises the sequences represented by SEQ ID NO: 45 and SEQ ID NO: 46;
a twenty-fourth primer pair comprises the sequences represented by SEQ ID NO: 47 and SEQ ID NO: 48;
a twenty-fifth primer pair comprises the sequences represented by SEQ ID NO: 49 and SEQ ID NO: 50;
a twenty-sixth primer pair comprises the sequences represented by SEQ ID NO: 51 and SEQ ID NO: 52;
a twenty-seventh primer pair comprises the sequences represented by SEQ ID NO: 53 and SEQ ID NO: 54;
a twenty-eighth primer pair comprises the sequences represented by SEQ ID NO: 55 and SEQ ID NO: 56;
a twenty-ninth primer pair comprises the sequences represented by SEQ ID NO: 57 and SEQ ID NO: 58;
a thirtieth primer pair comprises the sequences represented by SEQ ID NO: 59 and SEQ ID NO: 60;
a thirty-first primer pair comprises the sequences represented by SEQ ID NO: 61 and SEQ ID NO: 62;
a thirty-second primer pair comprises the sequences represented by SEQ ID NO: 63 and SEQ ID NO: 64;
a thirty-third primer pair comprises the sequences respresented by SEQ ID NO: 65 and SEQ ID NO: 66;
a thirty-fourth primer pair comprises the sequences respresented by SEQ ID NO: 67 and SEQ ID NO: 68;
a thirty-fifth pair comprises the sequences respresented by SEQ ID NO: 69 and SEQ ID NO: 70;
a thirty-sixth primer pair comprises the sequences respresented by SEQ ID NO: 71 and SEQ ID NO: 72; and

(C) comparing the genotype obtained from the tested Phalaenopsis variety with a known database of the Phalaenopsis genotype to identify the the tested Phalaenopsis variety.

10. The method as claimed in claim 9, wherein each of the microsatellite primer pair is a set of the primer pair sequences, the primer pair sequences comprise: any one nucleotide sequence selected from the group consisting of the first primer pair to the thirty-sixth primer pair.

11. The method as claimed in claim 9, wherein each of the microsatellite primer pair is a sequence set of the primer pair, the primer pair sequences are selected from the group consisting of the first primer pair to the thirty-sixth primer pair.

12. The method as claimed in claim 9, wherein at least one microsatellite primer pair is used to detect the genomic DNA of the tissue sample via a polymerase chain reaction (PCR).

13. The method as claimed in claim 9, wherein the at least one microsatellite primer pair comprises: a forward microsatellite primer and a reverse microsatellite primer.

14. The method as claimed in claim 13, wherein the 5' end of the forward microsatellite primer is labeled with a fluorescent reagent.

15. The method as claimed in claim 14, wherein the fluorescent reagent is HEX, FAM, or NED.

16. The method as claimed in claim 13, wherein the 5' end of the reverse microsatellite primer is labeled with a fluorescent reagent.

17. The method as claimed in claim 16, wherein the fluorescent reagent is HEX, FAM, or NED.

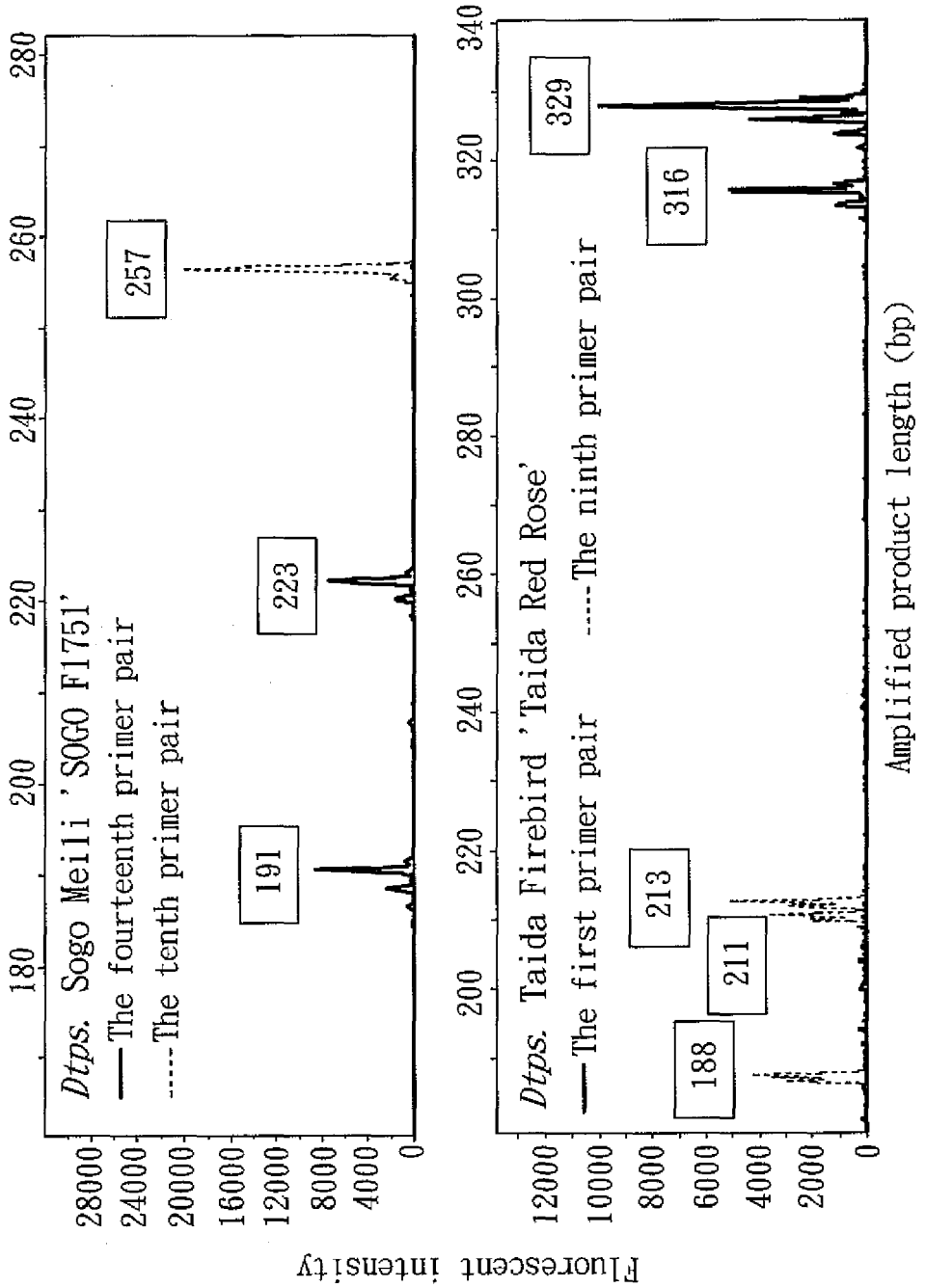
Dtps. Sogo Meili 'SOGO F1751'
The fourteenth primer pair
The tenth primer pair
191
223
257
Dtps. Taida Firebird 'Taida Red Rose'
The first primer pair
The ninth primer pair
188
211
213
316
329
Fluorescent intensity
Amplified product length (bp)

FIG. 1

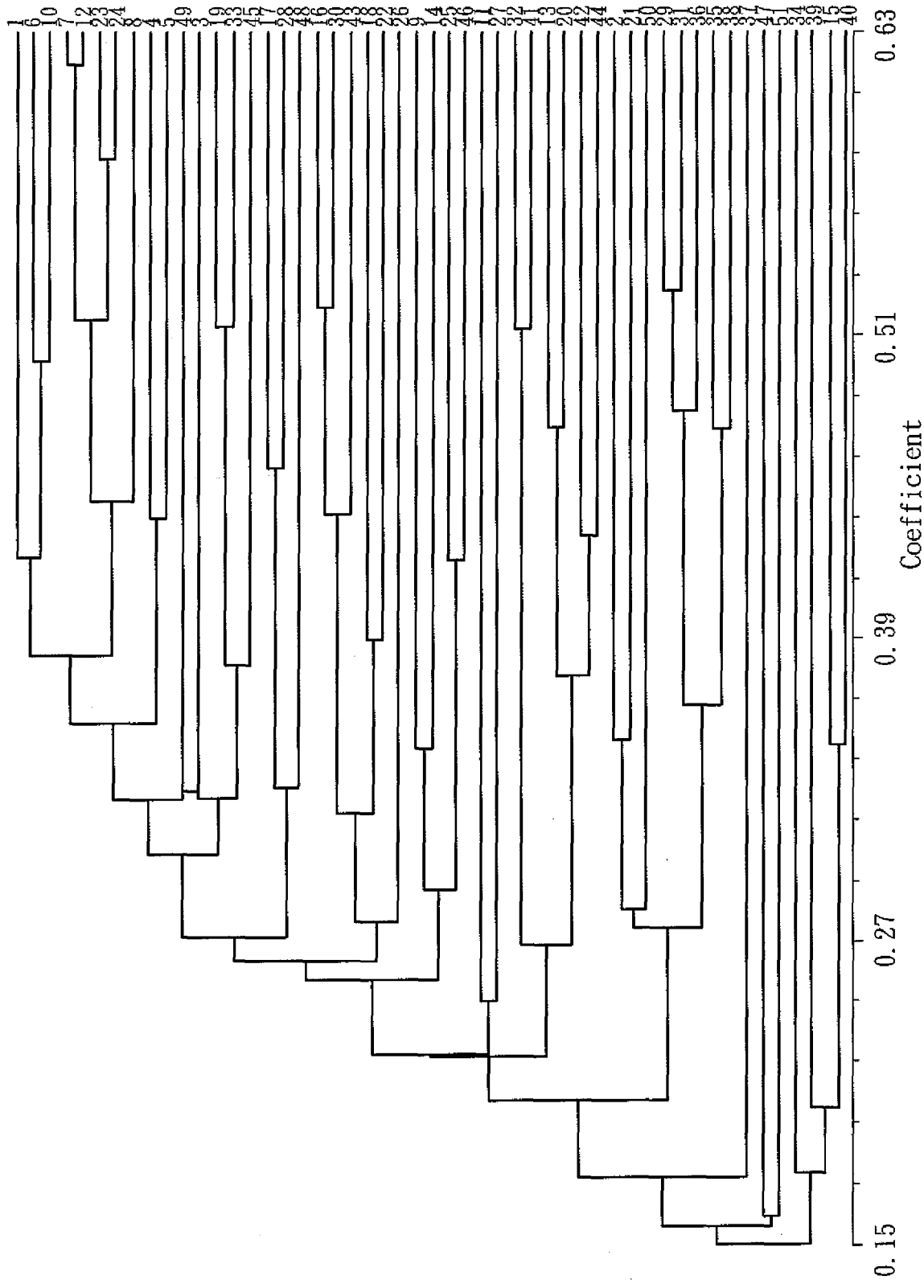
1 6 10 7 12 23 24 8 4 5 49 3 19 33 45 17 28 48 16 30 43 18 22 25 9 14 15 46 11 27 32 41 13 20 42 44 2 21 50 29 31 36 35 38 37 47 51 34 39 15 40
0.63
0.51
0.39
0.27
0.15
Coefficient

FIG. 2

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

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